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The Biology of Fungi Associated with Root Rot of Subterranean Clover in Victoria

By L. W. Burgess[1], H. J. Ogle[2], J. P. Edgerton[3], L. L. Stubbs[4] and P. E. Nelson[5]

Abstract: Ecological and pathogenicity studies suggest that decline of subterranean clover pastures in Victoria is due to root rot induced by a number of fungi. Many fungi were isolated from diseased roots collected in the field. However, only *Fusarium roseum* 'Avenaceum', *Fusarium roseum* ('Sambucinum'?) and *Pythium irregulare* proved to be very pathogenic in tests with seedlings. *Fusarium oxysporum*, *Fusarium roseum* 'Culmorum' and *Fusarium roseum* 'Gibbosum' were commonly associated with diseased roots but were not very pathogenic in these particular tests.

INTRODUCTION

Subterranean clover, *Trifolium subterraneum* L., is an annual species which has become the most important pasture legume in temperate regions of southern and eastern Australia (Powell 1970). Although subterranean clover is suitable to many localities, the occurrence of patchiness or decline has been reported in Victoria (Anon. 1960), South Australia (Ludbrook, Brockwell & Riceman 1953) and Western Australia (Shipton 1967). Stand deterioration is a widespread problem in many types of pastures and the local problem will be considered in context with research carried out elsewhere on similar problems. There is at present no satisfactory review of literature on the subject.

Deterioration of stands of temperate pasture legumes are reported in England as early as 1669 and in North America as early at 1747 (Fulton & Hanson 1960). Kilpatrick and Hanson (1950) estimated losses of 39-52 per cent in first year stands of red clover, *Trifolium pratense* L., in Wisconsin while Smith (1950), also working in Wisconsin, found that 60 per cent of red clover plants that survived their first summer failed to survive their first winter and that a large proportion of those remaining died during the following summer. In Ohio, stand losses of 95 per cent in a single growing season have been reported in individual clover fields (Selby & Thomas 1921). No estimates of losses in Australian pastures are available.

Several factors contribute to stand deterioration, but disease, particularly root and crown rot, has been a major factor (Fergus & Valleau 1926, Kreitlow & Hanson 1950, Crall 1951, Hanson 1953). In affected areas, plants are stunted and reddish-purple in colour. They may eventually die leaving a bare patch or they may recover during favourable weather. Roots of affected plants are rotted to various degrees and there is often marked discoloration of the primary root. This discoloration may extend into the crown of the plant.

Overseas, a large number of fungi has been identified on, or isolated from, affected plants (Cormack 1937a, 1937b, Buchholtz & Meredith 1938, Cherewick 1948, Staten & Leyendecker 1949, Kilpatrick & Hanson 1950, Kreitlow & Hanson 1950, Crall 1951, Hanson & Allison 1951, Hawn & Cormack 1952, Erwin 1954a, 1954b, Fulton & Hanson 1954, 1960, Kilpatrick, Hanson & Dickson 1954a, 1954b, McDonald 1955, Bushong & Gerdemann 1959, Graham & Newton 1959, Kilpatrick 1959, Leach 1959, Kainski 1960, Jenkins & Lindberg 1961, Kilpatrick & Dunn 1961, Leach, Dickason & Gross 1963, Schmitthenner 1964, Johnson & Morgan 1965, Willis 1965, O'Rourke & Millar 1966, Aubé & Des-

[1] Department of Agricultural Botany, University of Sydney, N.S.W., 2006, Australia.
[2] Plant Pathology Branch, Department of Primary Industries, Brisbane, Queensland, 4000, Australia.
[3] c/o Agricultural Institute, Kosbad-Hill, District Thana, Maharashtra, India.
[4] School of Agriculture & Forestry, University of Melbourne, Parkville, Victoria, 3052, Australia.
[5] Department of Plant Pathology, Pennsylvania State University, 211 Buckhout Laboratory, University Park, Pennsylvania, 16802, U.S.A.

chênes 1967, Denis & Elliott 1967, Frosheiser 1967, Johnson & Keeling 1969).

Fusarium oxysporum (Schlecht.) Snyder et Hansen has generally been most frequently isolated and has been shown to cause crown and root rot of lucerne, *Medicago sativa* L. (McDonald 1955), red clover (Kreitlow & Hanson 1950, Fulton & Hanson 1954, 1960, Kilpatrick, Hansen & Dickson 1954b) and wilting of lucerne (Weimer 1928, Jenkins & Lindberg 1961, Armstrong & Armstrong 1965) and red clover (Crall 1951) as well as pre- and post-emergence damping-off of legume seedlings (McDonald 1955, Kainski 1960). However, Erwin (1954b) and Schmitthenner (1964) reported that isolates of *F. oxysporum* from lucerne were not pathogenic when reinoculated onto lucerne.

Fusarium solani (Mart.) Synder et Hansen, another species commonly isolated from the roots of legumes showing decline symptoms, can cause severe damage to roots of red clover (Kilpatrick, Hanson & Dickson 1954b) and lucerne (Staten & Leyendecker 1949, McDonald 1955). *F. solani* also caused reductions in stands of red clover (Crall 1951).

Forms of *F. roseum* (Link) Snyder et Hansen have also been frequently isolated from diseased legumes and have been reported to be very pathogenic to seedlings (Erwin 1954a, McDonald 1955, Graham, Sprague & Robinson 1957, Kainski 1960) but not to plants beyond the seedling stage. However, Cormack (1937b) and Hawn and Cormack (1952) reported that *F. roseum* 'Avenaceum' was one of the most pathogenic species they tested. Similarly, Hawn and Cormack (1952) and McDonald (1955) found *F. roseum* 'Gibbosum', their most frequently isolated species, very pathogenic, although Cormack (1937b) considered it only weakly parasitic.

A number of other species of *Fusarium* have been isolated from roots and crowns of diseased legume plants. *F. moniliforme* (Sheld.) Snyder et Hansen caused reductions in stands of red clover (Crall 1951). *F. tricinctum* (Corda) Snyder et Hansen was the only species isolated from rotted lucerne roots by Lukezic, Bloom and Carroll (1969). It can cause complete wilting and death of several legume species and varieties within 72 hours of inoculation (Bolton & Nuttall 1968). Cormack (1937b) reported that *F. tricinctum* usually behaved as a weak pathogen on lucerne. *F. episphaeria* (Tode) Snyder et Hansen has also been reported associated with rotting lucerne roots (McDonald 1955).

A number of other fungi, such as *Rhizoctonia solani* Kuehn, *Gliocladium roseum* (Link) Thom, *Cylindrocarpon* spp. and species of *Pythium* and *Phytophthora* have been isolated from the roots and crowns of pasture legumes. *R. solani* is among the most important seedling pathogens of lucerne, causing pre- and post-emergence damping-off (McDonald 1955, Graham, Sprague & Robinson 1957, Schmitthenner 1964) as well as collar rot and crown bud rot (Chenewick 1948, Hanson & Allison 1951, Hawn & Cormack 1952, Benedict 1954, Kilpatrick, Hanson & Dickson 1954a, McDonald 1955). *G. roseum* can cause severe stunting but did not kill many plants in the pathogenicity tests reported by Kilpatrick, Hanson and Dickson (1954b). The pathogenicity of species of *Cylindrocarpon* to a number of species of legume was studied by Cormack (1937a) who found that *C. ehrenbergi* Wr. was decidedly more pathogenic than any other species tested. Species of *Pythium* generally cause damping-off of seedlings and do not attack advanced plants (Buchholz & Meredith 1938, Halpin, Hanson & Dickson 1952, 1954, Erwin 1954a, Kilpatrick, Hanson & Dickson 1954b, Halpin & Hanson 1958, Schmitthenner 1964). Species of *Phytophthora* cause either seedling damping-off (McDonald 1955, Johnson & Morgan 1968, Johnson & Keeling 1969) or root rot (Erwin 1954b), Bushong & Gerdemann 1959, Froshieser 1967).

There are some Australian reports on decline of pastures associated with the presence of various root-rotting fungi. In Western Australia, *F. oxysporum*, followed by *F. roseum* 'Avenaccum' were the organisms isolated most frequently from roots of diseased subterranean clover plants (Shipton 1967). These species, as well as *F. monoliforme*, were pathogenic to sterile seedlings grown in test tubes. *F. oxysporum* has recently been reported as the cause of lucerne decline in Western Australia (Marcley 1970). In South Australia, *R. solani*, *Pythium* spp., *Ophiobolus graminis* Sacc., *Corticium praticola* Kotila, *Helminthosporium* spp. and *Fusarium* spp. were among 300 isolates from rotted roots of subterranean clover plants from 'bare patch areas'(Ludbrook, Brockwell & Riceman 1953). However, only *R. solani* produced symptoms similar to those found in the field. In Victoria, *Fusarium* spp. and *Rhizoctonia* sp. were consistently isolated from diseased plants and laboratory tests indicated that the *Fusarium* spp. were responsible for the condition (Anon 1960). Recently, Kellock (1972) reported the isolation of *F. avenaceum* from both rotted roots and seeds of subterranean clover, and demonstrated that the fungus was highly pathogenic. Decline and establishment problems associated with root rots caused by species of *Pythium* and *Phytophthora* have also been reported (Teakle 1956, Andrew 1963, Purss 1965).

A wide range in the pathogenicity of isolates of many species has been reported (Cormack 1937a, 1937b, Benedict 1954, Kilpatrick, Hanson & Dickson 1954b, Fulton & Hanson 1960, Schmitthenner 1964, Denis & Elliott 1967). Furthermore, the relative importance of each species in the field is affected by the age of the stand, soil type, soil moisture and temperature (Cormack 1937a, 1937b, Kilpatrick, Hanson & Dickson 1954a, McDonald 1955, Graham, Sprague & Robinson 1957, Halpin & Hanson 1958, McGlohon 1959, Fulton & Hanson 1960, Kainski 1960, Willis 1965, Aubé & Deschênes 1967). Some authors have suggested that the fungi isolated from root rot lesions on legumes are weak parasites which become important only when the plants have been predisposed to infection by loss of food reserves with age, during the winter or with grazing (Young 1924, Fulton & Hanson 1954, O'Rourke & Millar 1966, Shipton 1967). Others have shown that the severity of root and crown rots is correlated with damage to roots by insects, such as the clover root borer, *Sitonia hispidula* (Fab.) (Graham & Newton 1959, Kilpatrick & Dunn 1961, Leach, Dickason & Gross 1963) and have suggested that mechanical injury to the roots due to the insects feeding facilitates the entry of the fungi. However, no direct correlation was found between *Pratylenchus* injury and root rot incidence (Leach, Dickason & Gross 1963). Furthermore, Chi, Childers and Hanson (1964) and Cormack (1937b) reported that *Fusarium* spp. can penetrate the roots of pasture legumes directly and do not require wounds for entry. Thus, the role of insect injury in the development of root rots of pasture legumes is uncertain. More recently, Khan and Banfield (1971) have suggested that failure of red clover in Massachusetts results from an interaction between red clover roots and certain viruses, *Fusarium* spp. and some insects.

The investigation reported in this paper was made to study the fungi associated with root rot of subterannean clover in Victoria and their pathogenicity.

FIELD SYMPTOMS

In the field plants affected by root rot were generally stunted and their foliage was usually reddish-purple to reddish-brown in colour. Root systems were rotted to various degrees and brown to black lesions could be observed at the junction of the lateral and tap root, along the lateral roots and at the root tip (Pl. 1 A, B, C). There was often a marked discoloration (usually reddish-brown) of the stele of affected lateral and tap roots. This discoloration frequently extended into the crown (Pl. 1, C). The affected root systems were often completely necrotic and it was not unusual to see lateral root proliferation from the tap root immediately beneath the crown on plants where the tap root was entirely rotted (Pl. 1, B).

Similar symptoms have been observed in young subterranean clover seedlings which were damping-off (Pl. 1, D). Diseased seedlings may also have a 'water-soaked' appearance which may be accompanied by a light brown discoloration of the cortical tissues of the root.

EXPERIMENTAL METHODS

(1) *Isolations from diseased roots*

Diseased root systems of subterranean clover plants were carefully removed from the soil and taken to the laboratory where they were washed and cultured on various media. The roots were carefully examined before culturing and notes were taken of disease symptoms. Approximately half were surface sterilized before culturing (1-2 min. in 0·1 per cent mercuric chloride in 10 per cent ethyl alcohol followed by four washings in sterile water) and all roots were 'damp-dried' between sterile paper tissues before culturing. The majority of the root systems were sampled from the Mt. Derrimut Field Station of the University of Melbourne. The remainder were sampled from various areas in northern and north-western Victoria. The origin of each sample was recorded using the co-ordinates of a 1:100,000 map of the series based on the Australian Map Grid.

(2) *Baiting method*

Diseased subterranean clover roots collected at Mt. Derrimut were washed, ground in a Waring blender and mixed with a pasteurised potting mix to give approximately 5 per cent inoculum by volume. Twenty-five *Rhizobium*-treated Bacchus Marsh subterranean clover seeds were then planted in pots containing the inoculated soil. Six pots were kept at each of the temperatures 10, 15, 20 and 25°C in a CSIRO unit phytotron (type B cabinets). Two pots were removed from each temperature so that a sampling was carried out when 50 per cent of the seedlings had emerged, when 50 per cent of the seedlings had the first trifoliate leaf unfolded and at 10 days after the second sampling.

At each sampling, the seedlings were removed from the two pots and bulked. Six seedlings were selected at random for serial culturing. The root systems were thoroughly washed under a tap water spray and then washed in five changes of sterile water. Segments 2 mm long were removed from the root cap, 1 cm above the root cap, 2 cm above the root cap, half way up the root and 1 cm below the soil surface. These

were then plated serially on three media—potato dextrose agar (PDA), a selective medium for *Fusarium* (Toussoun & Nelson 1968) and a selective medium for *Pythium* and *Phytophthora* (Tsao & Ocana 1969) so that two roots were cultured on each medium. The plates were incubated at room temperature and each colony that developed was subcultured on a PDA slope for subsequent identification.

(3) *Technique for soil Fusarium analyses*

All plant material was removed and the soil sample was then ground in a mortar and pestle. A 1 gm sub-sample was placed in 100 ml of sterile 0·1 per cent water agar. The soil suspension was shaken for 1 hour before the final dilutions to 1:500 to 1:3,000 were made. One ml of the final dilution was spread evenly over the surface of approximately 25 ml of the selective medium for *Fusarium* in 90 x 15 mm Petri dishes. Ten plates were made for each dilution of each soil sample. The medium was prepared so there was time for the plates to dry for 4-5 days before use. If this was not done, bacterial contamination became severe. The plates were incubated at room temperature (20°C) for 4-7 days. The dilution which gave a good, even distribution of colonies on the plates was kept. Each colony type on these plates was then numbered and the number of colonies of that type counted and recorded. Single conidium cultures were made from a single colony of each type before the final identification was made. The estimate of the number of propagules per gram of soil was made from the colony counts.

(4) *Pathogenicity tests*

The isolates used in the pathogenicity tests were representatives of the following species: *F. roseum* 'Avenaceum', *F. roseum* 'Culmorum', *F. roseum* ('Sambucinum'?), *F. roseum, F. oxysporum, F. solani, Pythium irregulare* Buisman, *Pythium aquatile* Hohnk, *Rhizoctonia* sp. The isolates were from subterranean clover roots with the exception of *F. solani* which was isolated from soil. The roots and soil were obtained from Field 8 at Mt. Derrimut.

The *Fusarium* isolates were maintained on PDA slopes using single conidium transfers. The other species were also maintained on PDA slopes and transferred by hyphal tips. All cultures were incubated at room temperature (25 ± 4°C) in diffuse daylight.

Inoculum was prepared by growing the fungi on a sand-chaff-maizemeal medium in preserving jars (Fowler No. 31) of 800 ml capacity. The medium in each jar consisted of 250 cc (approximately 400 g) of fine sand, 250 cc of cereal chaff containing approximately 5 per cent oat grain and 0·5 per cent wheat grain and 12 g of maizemeal. Each jar was shaken thoroughly to mix the contents before 125 ml of distilled water were added. Stainless steel lids, with 9 holes 0·5 cm in diameter, were used to permit aeration but at the same time limit drying through evaporation. To prevent contamination a layer of cotton wool enveloped in cotton gauze was placed over the lid of each jar and held down by the heat-resistant rubber rings supplied with the jars. The medium was autoclaved at 15 p.s.i. for 30 min. *Fusarium* isolates were added to the cooled medium as spore suspensions and the other species as 1 cm squares (4 per jar) of young colonies growing on PDA in Petri dishes. After inoculation, the jars were incubated at room temperature for 20 days. The daily maximum and minimum temperatures during this time were within the range 20-28°C. The inoculum was then used to amend soil (pasteurised by steam-air treatment for 30 min. at 82°C) in the ratio 4:1 (soil: inoculum) by volume. The control soil was amended with inoculum which had been exposed to propylene oxide. This involved the addition of 4 cc of propylene oxide (cooled to 0°C) to each jar of inoculum which was then sealed for 24 hours. These jars were opened for a further 24 hours in a fume cupboard for the gas to dissipate.

The soil used (pH 5) was collected from the top 15 cm of Field 8 at Mt. Derrimut. As it was heavy textured, it required breaking up in a soil shredder and moistening with a fine spray of water before pasteurising.

The amended soil was weighed into plastic buckets (17 cm tapering to 14 cm in diameter and 15 cm deep) and inoculated with a clover strain of *Rhizobium*. Three replicate buckets were used. Fifty seeds of Mt. Barker subterranean clover were sown in each bucket at a depth of 1 cm using a template. The moisture content of the amended soil was adjusted to field capacity after sowing and maintained at that level during the test. The buckets were kept in a cooled glasshouse and located at random on the benches. Glasshouse air temperatures during the test were within the range 15-30°C and soil temperatures in the root zone were in the range 17-30°C.

Twenty days after sowing, the plants were carefully removed from the soil and washed under a fine mist spray. Each plant was then rated for the degree of damage to its root system and hypocotyl according to a zero to five index scale as follows:

0—no visible lesions on roots
1—one or two small lesions (less than 0·5

cm long) on tap root or laterals, including root tip necrosis
2—more necrosis than 1 but less than 3
3—more than 2 healthy laterals above necrotic section of tap root but at least half the lower tap root with extensive necrosis
4—severe root damage—only 1 or 2 healthy laterals above necrotic section of tap root;
5—plant dead or tap root completely necrotic

The mean of the ratings of all plants in a pot was taken as the disease severity estimate for that pot. Representative necrotic roots from the various pathogenicity tests were cultured to determine the fungi present.

RESULTS

The following fungi were isolated from diseased subterranean clover roots: *F. moniliforme, F. oxysporum, F. roseum, F. roseum* 'Avenaceum', *F. roseum* 'Culmorum', *F. roseum* 'Gibbosum', *F. roseum* 'Graminearum', *F. solani, F. tricinctum, Cylindrocarpon* spp., *Gliocladium roseum, Helminthosporium* sp., *Mortierella* sp., *P. irregulare, P. aquatile, Pythium* sp., *Rhizoctonia* sp. and other unidentified genera. The relative frequency of isolation of these fungi is listed in Table 1.

The species of fungi isolated from 'bait' roots of subterranean clover seedlings grown in pasteurised soil amended with naturally infected clover roots are recorded in Table 2. On the selective medium for *Phytophthora* and *Pythium, P. irregulare* was predominant. The less frequently isolated species were not identified to species level.

The species of Fusarium isolated from the soil samples taken under subterranean clover stands and the estimated number of propagules per gram of soil of each are given in Table 3.

The relative severity of root rot in Mt. Barker subterranean clover seedlings caused by the *Fusarium* spp. tested is shown in Fig. 1. Species causing root rot induced localized or spreading lesions, brown to black in colour. Affected cortical tissue frequently developed a water-soaked appearance before becoming brown to black in colour. The stele of affected roots was usually darkened with a reddish-brown discoloration similar to that in rotted roots collected in the field. The cotyledons and leaves on severely affected seedlings wilted rapidly following collapse of the root system.

The symptoms resulting from infection by *Pythium* were similar to those described above. This fungus, however, did not seem to induce the discoloration of the stele. The data on symptom severity from the tests involving *Pythium* spp. and *Rhizoctonia* sp. are given in Fig. 2.

TABLE 1

Relative frequency of isolation of fungi from diseased subterranean clover roots

Species	*Relative frequency of isolation*
Fusarium moniliforme	+*
F. oxysporum	+++
F. roseum	++
F. roseum 'Avenaceum'	+
F. roseum 'Culmorum'	+++
F. roseum 'Gibbosum'	++
F. roseum 'Graminearum'	+
F. solani	+
F. tricinctum	+
Cylindrocarpon spp.	+
Gliocladium sp.	+
Helminthosporium sp.	+
Mortierella sp.	+
Pythium spp.	+
P. irregulare	+++
P. aquatile	+
Rhizoctonia sp.	+

* An arbitrary scale: + indicates occasional isolation; ++ indicates regular isolation in low numbers; +++ indicates frequent isolation.

TABLE 2

Species of fungi isolated from 'bait' roots of subterranean clover seedlings grown at four temperatures in pasteurised soil, amended with diseased subterranean clover roots

Soil Temperature °C	*Isolation Medium*	
	Potato dextrose agar	*Selective medium for Fusaria*
25	*F. oxysporum* (2)* *F. roseum* (6)** *F. roseum* 'Avenaceum' (2) *Dendrodochium* sp. (1)	*F. oxsporum* (1) *F. roseum* (3) *F. roseum* 'Avenaceum' (3) *Dendrodochium* sp. (2) *Pythium* sp. (1)
20	*F. roseum* (2) *F. roseum* 'Avenaceum' (3) *Cylindrocarpon* sp. (1) *Dendrodochium* sp. (1)	*F. oxysporum* (1) *F. roseum* (3) *F. roseum* 'Avenaceum' (1) *Cylindrocarpon* sp. (3) *Dendrodochium* sp. (1)
15	*F. roseum* (1) *Torula* sp. (1)	*F. roseum* (2) *F. roseum* 'Avenaceum' (1) *Cylindrocarpon* sp. (1)
10	*Torula* sp. (1)	*F. roseum* (2) *Cylindrocarpon* sp. (1) *Dendrodochium* sp. (1)

* Number in parenthesis is the total of the colonies of the fungus isloated at three sampling times from all root segments.
** Includes 'Culmorum', 'Gibbosum' and other types.

FIG. 1—The relative severity of root rot of Mt. Barker subterranean clover seedlings caused by species of *Fusarium*, based on an arbitrary disease severity scale of 0 — 5; 0 = no visible lesions on roots; 5 = competely necrotic tap-root. Black bars—inoculated; hatched bars—uninoculated.

TABLE 3

Fusarium spp. isolated from soil samples taken under subterranean clover stands in Victoria. (Number of propagules per gram of soil x 10^3)

Soil sample number	Map Reference	*F. oxysporum*	*F. solani*	*F. episphaeria*	*F. moniliforme*	*F. roseum*	*F. roseum* 'Gibbosum'
70-7-1 V	7822	8·5†	1·0				0·5
* 70-7-3 V	7822	17·0		0·5			
‡ 70-7-4 V	7822	5·0	1·0				
70-7-6 V	7822	5·0	0·5				
‡ 70-7-7 V	7822	5·0				0·5	
70-7-8 V	7822	6·5	0·5		0·5		
‡ 70-7-9 V	7822	3·5			2·0		1·0
‡ 70-7-10 V	7822	9·0			0·5		0·5
‡ 70-8-19 V	7124	8·0			0·5		
‡ 70-8-23 V	7124	7·0	1·0				
‡ 70-8-30 V	7925	3·0			3·5		1·4
‡ 70-8-31 V	7225	8·0	2·0				
70-8-32 V	7225	0·5	0·5				0·5
70-8-33 V	7925	15·5	4·0				
‡ 70-8-36 V	7724	0·5					
‡ 70-8-37 V	7823	1·0					0·5

* Samples which are bracketed are from adjacent areas within 500 ft of each other.

† Estimated number of propagules per gram of soil × 10^3.

‡ Soil from areas where root rot of subterranean clover is known to occur.

DISCUSSION

The symptoms of root rot of subterranean clover observed in various areas of Victoria were similar to those described in Western Australia (Shipton 1967) and indeed similar to those shown by root rot affected temperate legumes in other countries. The species of fungi isolated from diseased roots correspond to those reported previously.

F. oxysporum was frequently isolated from diseased roots and soil. However, it was not commonly isolated from the clover seedling roots used as baits. Furthermore, isolates of *F. oxysporum* were less pathogenic than previous reports suggested (Kilpatrick, Hanson & Dickson 1954a, McDonald 1955, Anon. 1960, Kainski 1960, Shipton 1967, Marcley 1970). These observations suggest that it is a secondary invader of diseased roots. Thus, the 'bait' technique used in this study offers a possible method for differentiating primary from secondary invaders before time-consuming pathogenicity tests are undertaken.

F. solani also proved less pathogenic than expected from previous reports (Crall 1951, Kilpatrick, Hanson & Dickson 1954a, 1954b, McDonald 1955, Kainski 1960) and is probably also only a secondary invader.

Isolates of *F. roseum* 'Avenaceum' were generally most pathogenic. This is in accord with the findings of Cormack (1937b), Hawn and Cormack (1952) and Kellock (1972). It is interesting that although *F. roseum* 'Avenaceum' was not commonly isolated from diseased roots from the field or from soil, it was consistently isolated using the 'bait' technique. This suggests that it may be a primary pathogen which is not easily isolated on culture media in competition with other fungi. The development of an improved direct isolation procedure for this fungus would greatly facilitate the study of its distribution in pasture soils.

Other *F. roseum* types were frequently isolated from diseased roots and from 'bait' roots which indicates that they may also be primary pathogens. An isolate of *F. roseum* ('Sambucinum'?) was quite pathogenic. *F. roseum* 'Culmorum' was not as pathogenic as expected from the results reported by Cormack (1937b). He did, however, observe that the pathogenic activity of *F. roseum* 'Culmorum' was suppressed at lower temperatures. This may account for its non-pathogenicity in our tests.

The fact that *P. irregulare* was frequently isolated from 'bait' roots suggested that it, too, is a primary pathogen. The pathogenicity studies indicated that it can cause significant root rot and damping-off of subterranean clover seedlings. It was frequently isolated from diseased roots (from both mature and immature plants) from the field and particularly from small lesions along young lateral roots and at the root tips. Thus, *P. irregulare* may have a detrimental effect on the growth of the plants through necrosis of the feeder rootlets as discussed by Wilhelm (1959).

Rhizoctonia sp. was not significantly pathogenic, in contrast to the results reported previously (Ludbrook, Brockwell & Riceman 1953, Benedict 1954, McDonald 1955, Kainski 1960). However, only one isolate was tested and it is well known that *Rhizoctonia* isolates vary in pathogenicity (Benedict 1954, Kainski 1960). The status of the remaining species as pathogens is uncertain but their low overall frequency of isolation using the three procedures suggests that they are not important.

Our results suggest that root rot of suberranean clover is caused by a complex of pathogens, since we have shown that three species representing two genera are strongly pathogenic. At this stage, not enough is known about the relative roles of these fungi or of possible interactions between them for

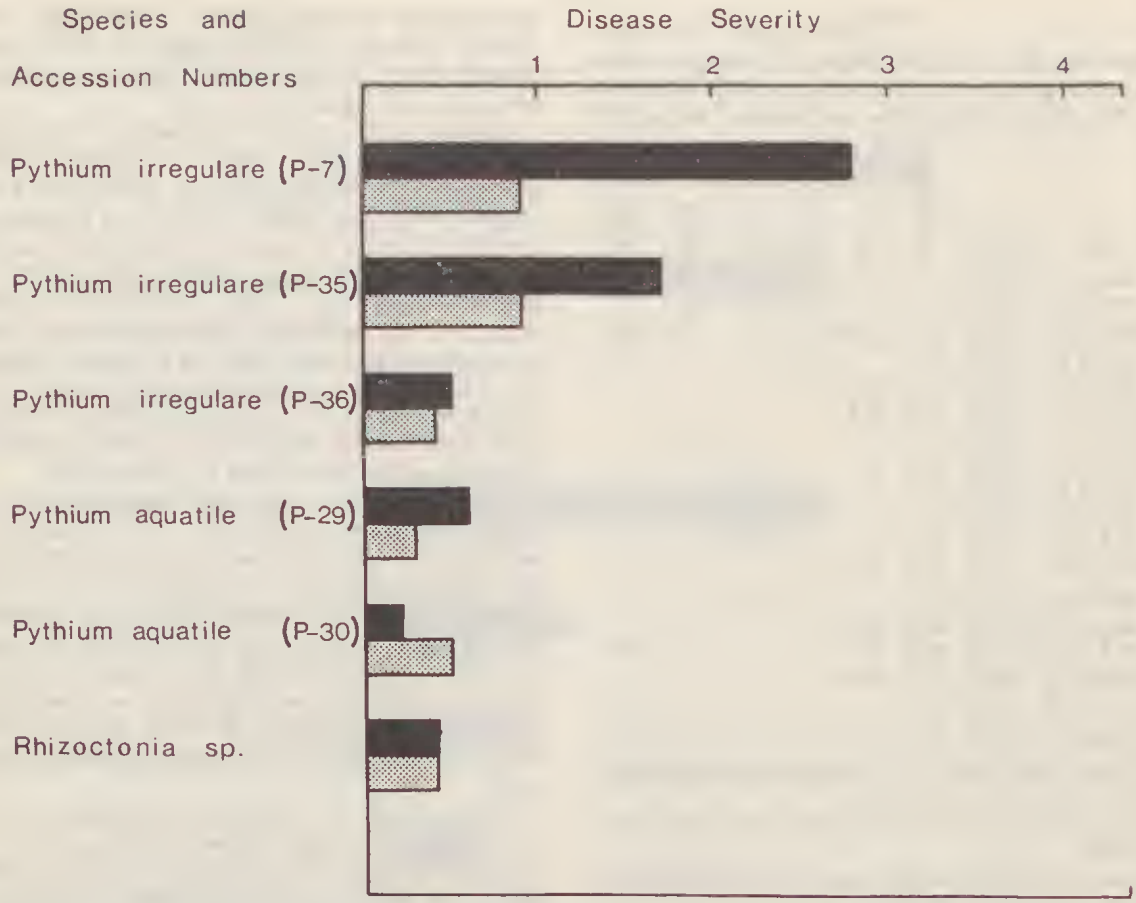

FIG. 2—The relative severity of root rot of Mt. Barker subterranean clover seedings caused by species of *Pythium* and a *Rhizoctonia sp.*, based on an arbitrary disease severity scale of 0 — 5; 0 = no visible lesions on roots; 5 = completely necrotic tap-root. Black bars—inoculated; hatched bars—uninoculated.

any definite conclusions to be drawn as to their relative importance as pathogens.

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REFERENCES

ANDREW, W. D., 1963. Susceptibility to damping-off in seedlings of *Medicago denticulata* Willd. and *M. minima* (L.) Bart. during the early post-emergence period. *Aust. J. biol. Sci.* 16: 281.

ANON., 1960. *Victorian Department of Agriculture, Report of the Biology Branch* 1.

ARMSTRONG, G. M. & ARMSTRONG, J. K., 1965. Further studies on the pathogenicity of three forms of *Fusarium oxysporum* causing wilt of alfalfa. *Pl. Dis. Reptr.* 49: 412.

AUBÉ, C. & DÊSCHENES, J., 1967. Crown and root rots of alfalfa and red clover and the relative prevalence of associated fungi. *Ibid.* 51: 573.

BENEDICT, W. G., 1954. Stunt of clovers, caused by *Rhizoctonia solani*. *Can. J. Bot.* 32: 215.

BOLTON, A. T. & NUTTAL, V. W., 1968. Pathogenicity studies with *Fusarium poae*. *Can. J. Pl. Sci.* 48: 161.

BUCHHOLTZ, W. F. & MEREDITH, C. H., 1938. *Pythium debaryanum* and other *Pythium* species cause alfalfa seedling damping-off. *Phytopathology* 28: 4 (Abstract).

Bushong, J. W. & Gerdemann, J. W., 1959. Root rot of alfalfa caused by *Phytophthora cryptogea* in Illinois. *Pl. Dis. Reptr.* 43: 1178.

Cherewick, W. J., 1948. Two important diseases of alfalfa in Manitoba. *Phythopathology* 38: 5 (Abstract).

Chi, C. C., Childers, W. R. & Hanson, E. W., 1964. Penetration and subsequent development of three Fusarium species in alfalfa and red clover. *Ibid.* 54: 434.

Cormack, M. W., 1937a. *Cylindrocarpon ehrenbergi* Wr., and other species, as root parasites of alfalfa and sweet clover in Alberta. *Can. J. Res.* C 15: 403.

————, 1937b. *Fusarium* spp. as root parasites of alalfa and sweet clover in Alberta. *Ibid.* C 15: 493.

Crall, J. M., 1951. Wilt of red clover seedlings. *Phytopathology* 41: 7 (Abstract).

Denis, S. J. & Elliott, E. S., 1967. Decline of red clover plants infected with red clover vein mosaic virus and *Fusarium* species. *Ibid.* 57: 808.

Erwin, D. C., 1954a. Relation of *Stagonospora, Rhizoctonia* and associated fungi to crown rot of alfalfa. *Ibid.* 44: 137.

————, 1954b. Root rot of alfalfa caused by *Phytophthora cryptogea. Ibid.* 44: 700.

Fergus, E. N. & Valleau, W. D., 1926. A study of clover failure in Kentucky. *Kentucky Agric. Exp. Stn. Res. Bull.* 269: 139.

Frosheiser, F. I., 1967. *Phytophthora* root rot of alfalfa in Minnesota. *Pl. Dis. Reptr.* 51: 679.

Fulton, N. D. & Hanson, E. W., 1954. Pathogenicity of fungi isolated from diseased red clover roots in Wisconsin. *Phytopathology* 44: 489 (Abstract).

———— & ————, 1960. Studies on root rots of red clover in Wisconsin. *Ibid.* 50: 541.

Graham, J. H. & Newton, R. C., 1959. Relationship between root feeding insects and incidence of crown and root rot in red clover. *Pl. Dis. Reptr.* 43: 1114.

————, Sprague, V. C. & Robinson, R. R., 1957. Damping-off of Ladino clover and Lespedeza as affected by soil moisture and temperature. *Phytopathology* 47: 182.

Halpin, J. E. & Hanson, E. W., 1958. Effect of age of seedlings of alfalfa, red clover, ladino clover, and sweet clover on susceptibility to *Pythium. Ibid.* 48: 481.

Halpin, J. E., Hanson, E. W. & Dickson, J. G., 1952. Studies on the pathogenicity of seven species of *Pythium* on red clover seedlings. *Ibid.* 42: 245.

————, ———— & ————, 1954. Studies on the pathogenicity of seven species of *Pythium* on alfalfa, sweet clover, and Ladino clover seedlings. *Ibid.* 44: 572.

Hanson, C. H. & Allison, J. L., 1951. Studies on the nature and occurrence of stand depletion in alfalfa strains in North Carolina. *Agron. J.* 43: 375.

Hanson, E. W., 1953. Relative prevalence and severity of the diseases of forage legumes in Wisconsin, 1946-1952. *Pl. Dis. Reptr.* 37: 467.

Hawn, E. J. & Cormack, M. W. 1952. Crown bud rot of alfalfa. *Phytopathology* 42: 510.

Jenkins, J. H. & Lindberg, G. D., 1961. *Fusarium* wilt of alfalfa in Louisiana. *Ibid.* 51: 643 (Abstract).

Johnson, H. W. & Keeling, B. L., 1969. Pathogenicity of *Phytophthora megasperma* isolated from subterranean clover roots. *Ibid.* 59: 1279.

———— & Morgan, F. L., 1965. *Phytophthora* root rot and crown rot of alfalfa in the Yazoo-Mississippi delta. *Pl. Dis. Reptr.* 49: 753.

Kainski, J. M., 1960. Study of fungi involved in root rots and seedling diseases of birdsfoot trefoil. *Cornell Univ. Agric. Exp. Stn. Mem.* 369.

Kellock, A. W., 1972. A fungus that rots the roots of subterranean clover. *J. Agric.*, Victoria 70: 112.

Khan, H. A. & Banfield, W. M. 1971. Some factors involved in the failure of red clover in Massachusetts. *Phytopathology* 61: 129 (Abstract).

Kilpatrick, R. A., 1959. Fungi associated with red and white clover in New Hampshire. *Pl. Dis. Reptr.* 43: 1111.

———— & Dunn, G. M., 1961. Fungi and insects associated with deterioration of white clover taproots. *Crop Sci.* 1: 147.

———— & Hanson, E. W., 1950. Root and crown rots of red clover in Wisconsin. *Phytopathology* 40: 14 (Abstract).

Kilpatrick, R. A., Hanson, E. W. & Dickson, J. G., 1954a. Root and crown rots of red clover in Wisconsin and the relative prevalence of associated fungi. *Ibid.* 44: 252.

————, ———— & ————, 1954b. Relative pathogenicity of fungi associated with root rots of red clover in Wisconsin. *Ibid.* 44: 292.

Kreitlow, K. W. & Hanson, R. G. 1950. Role of *Fusarium* in loss of red clover stands. *Ibid.* 40:16 (Abstract).

Leach, C. M., 1959. A survey of root deterioration of *Medicago sativa* in Oregon. *Pl. Dis. Reptr.* 43: 622.

————, Dickason, E. A. & Gross, A. E., 1963. The relationship of insects, fungi, and nematodes to the deterioration of roots of *Trifolium hybridum* L. *Ann. appl. Biol.* 52: 371.

Ludbrook, W. V., Brockwell, J. & Riceman, D. S., 1953. Bare-patch disease and associated problems in subterranean clover pastures in South Australia. *Aust. J. agric. Res.* 4: 403.

Lukezic, F. L., Bloom, J. R. & Carroll, R. B., 1969. Development of *Fusarium* root rot and its effect on root and crown carbohydrate levels of clipped alfalfa plants grown in a gnotobiotic environment. *Phytopathology* 59: 1575.

McDonald, W. C., 1955. The distribution and pathogenicity of the fungi associated with crown and root rotting of alfalfa in Manitoba. *Can. J. agric. Sci.* 35: 309.

McGlohon, N. E., 1959. Survey of fungi associated with white clover stolons. *Pl. Dis. Reptr.* 43: 22.

Marcley, M. D., 1970. *Fusarium oxysporum* as a cause of lucerne decline in Western Australia. *Ibid.* 54: 1061.

O'Rourke, C. J. & Millar, J. L., 1966. Root rot and root microflora of alfalfa as affected by potassium nutrition, frequency of cutting, and leaf infection. *Phytopathology* 56: 1040.

Powell, S. C., 1970. Subterranean clover, our most important pasture legume. *J. Agri.*, Victoria 68: 274.

Purss, G. S., 1965. Diseases of lucerne. *Qd. Agric. J.* 91: 196-206.

Schmitthenner, A. F., 1964. Prevalence and virulence of *Phytophthora, Aphanomyces, Pythium, Rhizoctonia* and *Fusarium* isolated from diseased alfalfa seedlings. *Phytopathology* 54: 1012.

Selby, A. D. & Thomas, R. C., 1921. Impairment of clover seedlings reported. *Ohio agric. Exp. Stn. Mon. Bull.* 6: 90.

SHIPTON, W. A., 1967. Fungi associated with 'purple patch' of subterranean clover in Western Australia. *Aust. J. Sci.* 30: 65.

SMITH, D., 1950. Seasonal fluctuations of root reserves in red clover. *Pl. Physiol., Wash.* 25: 702.

STATEN, G. & LEYENDECKER, P. J., 1949. A root rot of alfalfa caused by *Fusarium solani*. *Pl. Dis. Reptr.* 33: 254.

TEAKLE, D. S., 1956. A lucerne root rot caused by *Pythium myriotylum*. *Qd. J. agric. Sci.* 13: 241.

TOUSSOUN, T. A. & NELSON, P. E., 1968. *A pictorial guide to the identification of Fusarium species according to the taxonomic system of Snyder and Hansen*. Pennsylvania State University Press, University Park and London, pp. 51.

TSAO, P. H. & OCANA, G., 1969. Selective isolation of species of *Phytophthora* from natural soils on an improved antibiotic medium. *Nature, London* 223: 636.

WEIMER, J. L., 1928. A wilt disease of alfalfa caused by *Fusarium oxysporum* var. *medicaginis* n. var. *J. Agric. Res.* 37: 419.

WILHELM, S., 1959. Parasitism and pathogenesis of root disease fungi. *In Plant Pathology Problems and Progress*: 1908-1958. Holton, C. S. et al. (Eds.) University of Wisconsin Press, Madison.

WILLIS, C. B., 1965: Root rot of red clover in Prince Edward Island and factors influencing the incidence of associated fungi. *Can. J. Pl. Sci.* 45: 369.

YOUNG, W. J., 1924. An Investigation of clover root rot. *Phytopathology* 14: 63 (Abstract).

DESCRIPTION OF PLATE 1

Subterranean clover roots with typical root-rot symptoms. A. Rotted tap root. B. Rotted tap root with lateral root proliferation above the rotted section. C. Tap roots, with root rot, sectioned longitudinally. D. Seedlings severely affected with root rot.